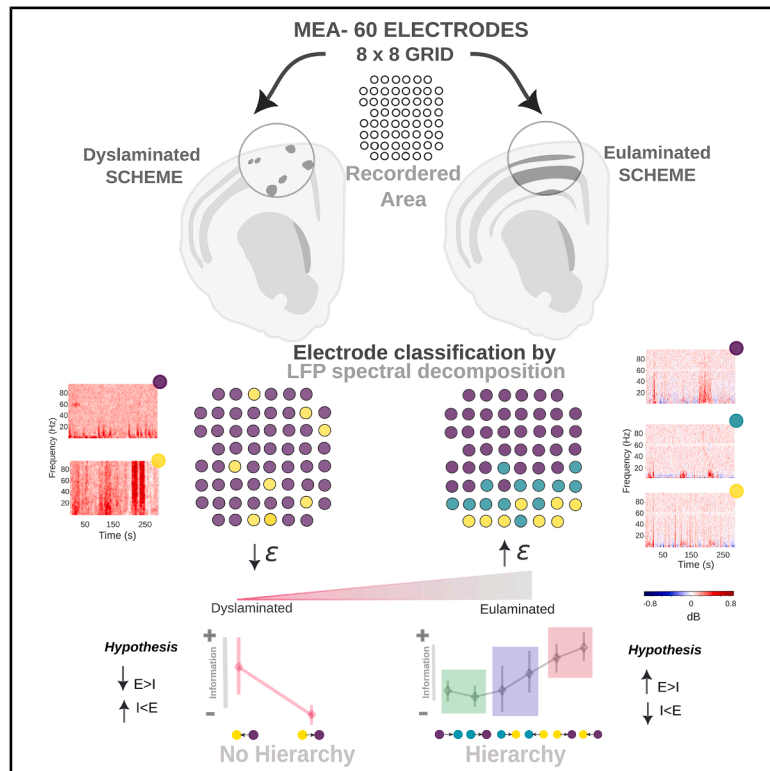


Assessing the degree of cortical dislamination through electrical pattern analysis

Graphical abstract



Authors

Ana Aquiles, Laura Pinedo, Mirelta Regalado, Hiram Luna-Munguia, Fernando Peña-Ortega, Luis Concha, Román Rossi-Pool

Correspondence

lconcha@unam.mx (L.C.), romanr@ifc.unam.mx (R.R.-P.)

In brief

Health sciences; Biological sciences; Neuroscience; Techniques in neuroscience

Highlights

- Field potential spectral decomposition extracts features to classify electrical activity
- Electrode classification describes functional alteration degree in cortical dyslamination
- Excitatory-inhibitory balance may relate to dysfunction within the dyslaminated cortex
- The degree of functional alterations may be linked to cytoarchitectural disarrangement



Article

Assessing the degree of cortical dislamination through electrical pattern analysis

Ana Aquiles,¹ Laura Pinedo,¹ Mirelta Regalado,¹ Hiram Luna-Munguia,¹ Fernando Peña-Ortega,¹ Luis Concha,^{1,*} and Román Rossi-Pool^{2,3,*}

¹Institute of Neurobiology, Universidad Nacional Autónoma de México, Campus Juriquilla, Querétaro, Mexico

²Institute of Cellular Physiology, Universidad Nacional Autónoma de México, Cognitive Neuroscience Department, Mexico City, Mexico

³Lead contact

*Correspondence: lconcha@unam.mx (L.C.), romanr@ifc.unam.mx (R.R.-P.)

<https://doi.org/10.1016/j.isci.2026.115942>

SUMMARY

Focal cortical dysplasia (FCD) is a leading cause of pharmacoresistant epilepsy in pediatric populations, yet its role in epileptogenesis remains incompletely understood. Recent findings suggest that hyperexcitability may originate in non-dysplastic peripheral areas rather than the malformation itself. However, given the significant variability across FCD cases, clarifying whether cytoarchitectural disorganization drives activity changes remains challenging. Using the carmustine-induced animal model, we investigated how varying degrees of cortical malformation influence neural dynamics. Local field potentials were recorded via multielectrode arrays during spontaneous activity and external perturbation. We introduced a novel metric to quantify spatial heterogeneity in signal organization and assessed its association with excitation-inhibition balance. Results show that alterations in signal structure correlate with the extent and distribution of cortical abnormalities, highlighting the functional relevance of cytoarchitectural variability. This work advances understanding of FCD-related network dysfunction and offers analytical tools with potential translational value for pre-surgical evaluation.

INTRODUCTION

Focal cortical dysplasia (FCD) is the leading cause of surgical intervention for pharmacoresistant epilepsy in pediatric patients.^{1,2} The occurrence of FCD in cases of focal epilepsy is roughly 5–25%. Yet, despite this prevalence, the relationship between FCD and epilepsy remains incompletely understood,¹ as not every cortical malformation progresses to epilepsy.³ It has been suggested that hyperexcitation in pediatric FCD type IIa may stem from the presence of balloon cells,⁴ which are primarily identified by their unique morphology. However, balloon cells do not trigger epileptic activity despite their highly abnormal appearance; rather, dysmorphic neurons and immature neurons—cytological hallmarks of FCD IIa—are the primary contributors to epileptic discharges.⁵ The combination of animal models and advanced analytical techniques has brought us closer to understanding the mechanisms of cortical dysplasias. Groundbreaking discoveries by Koh and collaborators,⁶ have challenged traditional views of cortical dysplasia, demonstrating that functional impairments may originate not within the primary malformation itself, but rather in surrounding non-malformed neurons. Their findings reveal that hyperexcitability often emerges from these ostensibly intact regions, while neurons within the malformed zones can remain hypoexcitable. The balance between excitation and inhibition is a fundamental concept to understand neural circuit deformation and brain dynamics alteration. These principles have been increasingly explored to

understand the emergence of epilepsy and other neurological conditions across various spatial and temporal scales,^{7,8} including during the pre-ictal activity, which has been widely regarded as a critical window into the mechanisms leading to seizure onset.^{9,10} Yet, despite growing interest in early electrical changes, the extent to which the degree of cortical delamination caused by cortical dysplasia contributes to functional alterations remains underexplored. In this work, we propose that spontaneous activity and, particularly, the local balance between excitation and inhibition, may play a key role in mediating how varying severities of malformation disrupt neural dynamics. To address the latter, we employed the carmustine model to simulate the cytoarchitecture of cortical dysplasia type IIa-like, as previously reported,^{11–17} this model is notable for its broad range of malformation severity, making it an effective representation of the morphological challenges associated with this condition. Importantly, since this model does not exhibit functional changes such as seizure activity,¹⁵ we opted to measure spontaneous electrical activity using (MEA), and the Mg^{2+} Free model^{18–22} to mimic the electrical currents evoked during epileptiform activity. Here, we decomposed the local field potential (LFP) spectrum to assess potential differences in signal structure based on cortical localization, and categorized those differences using frequency covariation. To quantify this categorization, we developed a metric that captures the heterogeneity of electrode groupings within each MEA recording. We then evaluated the aperiodic exponent in relation to an excitatory-inhibitory (E/I)



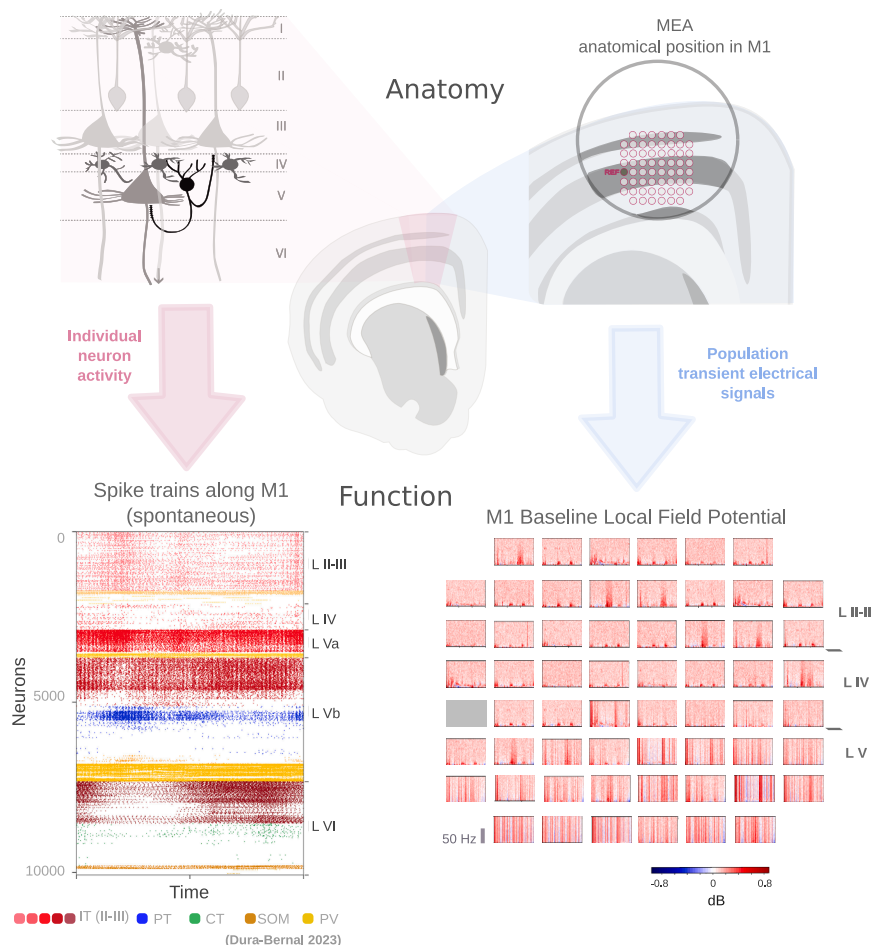


Figure 1. Hypothesis

Taking a similar approach to that of Dura-Bernal et al. (2023), who classified neuronal types based on neuronal firing patterns, we propose categorizing electrodes by their activity using temporal frequency covariations, as measured by LFP signals.

morphological features from FCD IIa,^{11,13,14} such as loss of cortical lamination and some other cell malformations (Figure 1). Also, we incorporated a model that induces epileptogenic conditions. The Mg^{2+} -Free model, previously reported and described,^{18,25,26} was employed to assess how the absence of Mg^{2+} ions affects current dynamics and how these changes relate to cortical disorganization. We compare signal differences in approximately the medial motor cortex (see Methods 10), where the MEA recorded activity spanning layer II-III to V (Figure 2). We first qualitatively examined the logarithmic power spectra (Figure 3A). Visual inspection of raw traces of untreated conditions (Figure 3B) across different states revealed noticeable differences in spontaneous and evoked activities. It is evident from the spectrograms, even without formal analysis, that the primary distinctions between the conditions become apparent during spontaneous activity.

balance metric,^{8,23,24} aiming to uncover a potential link with the degree of functional spatial sparsity in the categorization. We describe the flow of information between different classes of electrodes during spontaneous activity, which varies depending on the level of dyslamination. We also identified a common fluctuation band that spans various activity states—spontaneous, magnesium-free, and recovery. Finally, we monitored its information flow during temporal evolution in relation to functional electrode categorization. Through this work, we aim to deepen the understanding of dysfunction in cortical dysplasia while accounting for the variability inherent in these malformations, while also offering analytical approaches that may inspire and support the study of complex biological time series in other systems characterized by structural and functional variability.

RESULTS

Neural recordings from medial motor cortices during spontaneous activity, perturbation (Mg^{2+} -Free) and recovery

To describe the electrical activity differences between healthy tissue and with cortical disarrangement (i.e., Eulaminated and Dyslaminated, respectively), we evaluated M1 cortex in an animal model of cortical maldevelopment,¹⁵ which reproduces

Remarkably, these differences are much more pronounced during this resting period than during the other conditions. Nevertheless, it is also noteworthy that the appearance of both signals evolved differently during the perturbation challenge and continued during the recovery phase. The quantitative analysis of these observations is described in the following sections.

Categorization of electrodes and effects on the aperiodic exponent

Following the idea that a normal anatomical cortical array can lead to a functional structure, as shown by Dura-Bernal,²⁷ based on real and simulated spike train data, we first focused on the description of spontaneous activity (Figures 2, 3, and 4). We emphasized the categorization of electrodes within the MEA, using the distinct signal shapes associated with electrode depth (Figures 2 and 4A). We covary the frequency domain (60 points, from 0.5 to 100 Hz) over time for each electrode, and noted that the cumulative variance captured by the first modes (components) was greater in the Eulaminated condition than in the Dyslaminated one (Figure 4B). To further explore this result, we projected the first two modes on a comodulogram constructed from the spectrogram, ordered by electrode position in the MEA (Figure 4C), where we observed differences in frequency

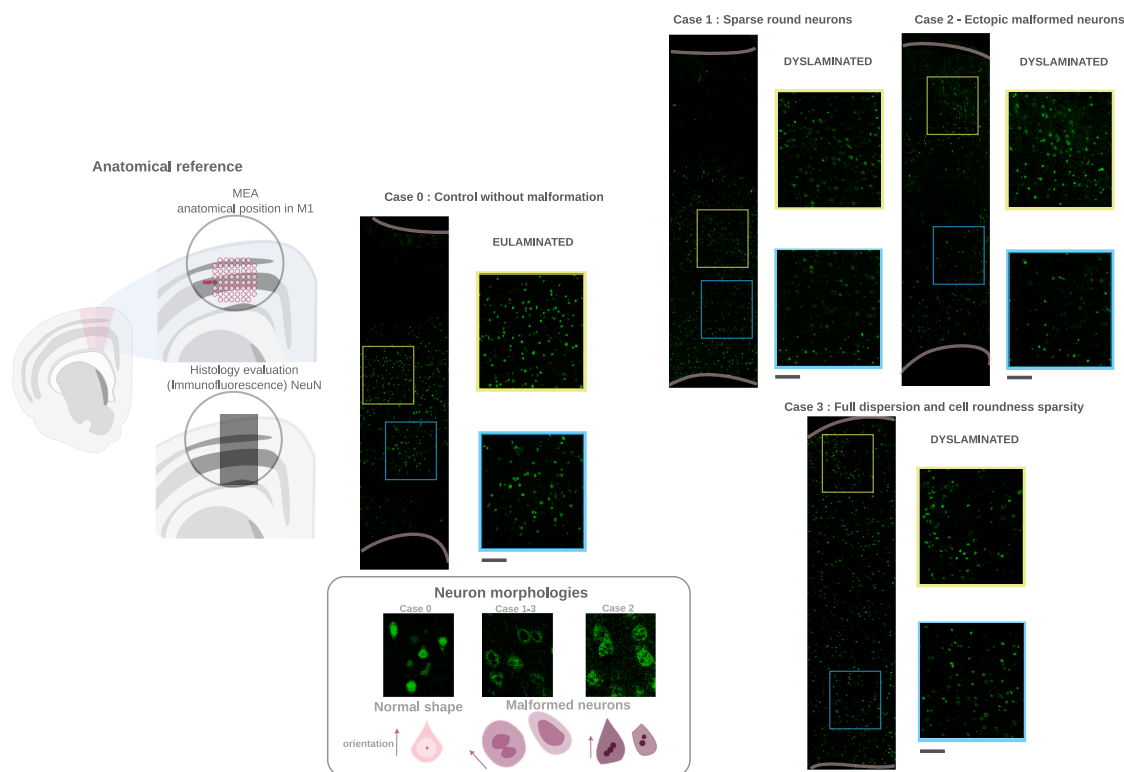


Figure 2. NeuN cortical characteristics analyzed among animals from the same litter

In the left panel, we present the anatomical reference for the MEA recording plane, along with a schematic of the histological evaluation. The right panel displays four different cases pertaining to cortical columns measuring (Width: 550 μm , Height: 2200 μm). The yellow and blue squares indicate specific zones where morphology is most evident, with a scale bar of 100 μm . Each column marks the upper limit (pia) and the lower limit (cingulum). Control pups are represented by case 0, which shows no malformations, while Cases 1–3 illustrate the variability of malformations induced by the BCNU model. The lower square provides a closer look at the differing neuron morphologies, highlighting that case 0 contains neurons with normal shape and orientation. In contrast, Cases 1–3 exhibit rounded neurons with cytoplasmic alterations and disorganized orientations, while Case 2 specifically shows malformed ectopic neurons. This zoomed-in section corresponds to a scale of 80 μm . Complementary results are shown in Figure S1.

range and weighted magnitude across Modes 1 and 2. Mode 1 shows that the initial electrodes exhibit stable frequency and weighting values, while later electrodes display an upward trend in these values. Additionally, the Dyslaminated projection identifies several electrodes that display distinct behavior compared to the others. In module 2, variations in the electrode spectrum are also observed. This observation motivated us to look for groups in the space projection of the first two components. We obtained a variation of groups and dispersion along the MEA architecture, which led us to develop a metric to describe the degree of sparsity of the clusters. We refer to this metric as ϵ , (see Methods 10), with values ranging from 0 to 1, where lower ϵ values indicate higher sparseness and less structured clustering, and higher values indicate the opposite (Figure 4D). Statistical analysis revealed significant differences in ϵ between the Eulaminated and Dyslaminated arrays, providing a measure to quantify the degree of disorder. Furthermore, based on previous results, these findings also highlight the range and variety of disorders, along with the histological validation of the same model.^{15,17} Consequently, we suggest a hypothetical link between the ϵ metric and cytoarchitecture, as illustrated in Figure 4D, bottom. As

depicted in our initial observations (Figure 3A), we next examined the aperiodic component found in each electrode of the MEAs to assess the observed variations. In Figure 3E, it is evident that the most extreme cases along the disorder gradient exhibit distinct power-law scale decay, based on the mean and variance of all measured electrodes. Interestingly, when evaluating the distribution of total aperiodic exponent values (Figure 4F), a bimodal function emerges, with a clear decline around the 0.6 value (sensitivity: 0.71, specificity: 0.53, and 95% CI for Accuracy: [0.612, 0.691]). Based on this observation, we classified the aperiodic exponents using 0.6 as a threshold and re-evaluated the data using the same four representative examples shown in Figure 4D to investigate whether the previously identified clusters were preserved. This analysis revealed that Dyslaminated arrays predominantly exhibit aperiodic exponent values higher than 0.6, whereas Eulaminated arrays show a balanced distribution of values both below and above this threshold. To assess how well these values are related to the actual level of disorder described by ϵ , we performed a traditional linear regression between the bimodal coefficient of each individual array distribution and its ϵ value (Figure 5A). However, we found no

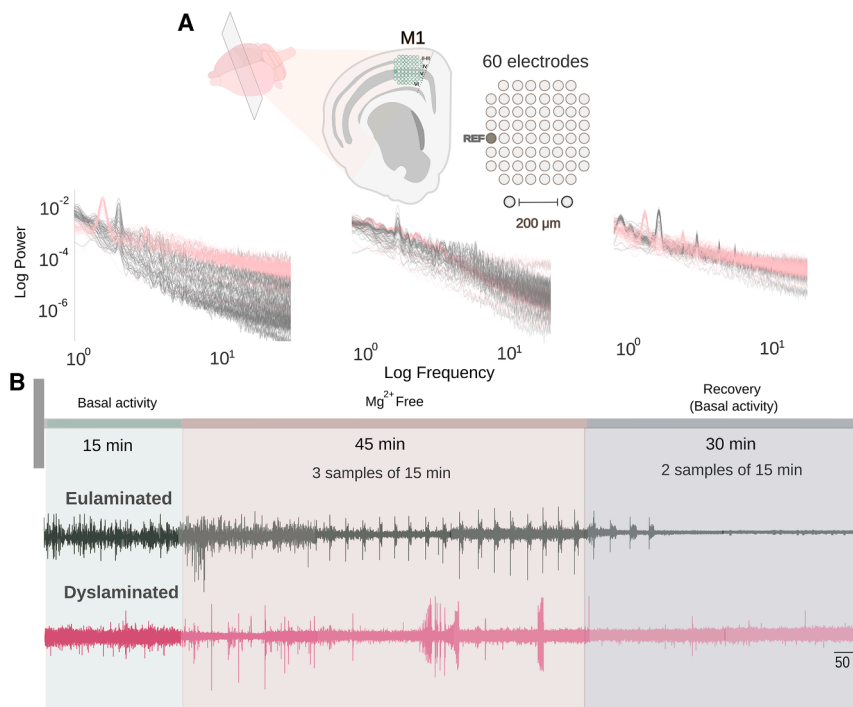


Figure 3. Overview. Multielectrode array recordings from normal (eulaminated) and dyslaminated cortices

(A) Spectral decomposition of spontaneous activity under three conditions in each of the 60 electrodes, for one control (gray) and one animal with dyslaminated cortex (pink).

(B) Exemplary time courses of spontaneous cortical activity under the three conditions. Each sample was about 15, 45, and 30 min per activity type (green - Basal activity, pale rose - Mg²⁺-Free, pale violet - Recovery). Examples were selected from the extreme cases of each condition. See also Figure 4.

significant correlation between them. Here, it is analyzed as an additional axis and not as an effect of the ϵ value. Nevertheless, as shown in both Figures 5A and 5B, a tendency toward a bimodal distribution is apparent, where there exist both a high and a low aperiodic values. Following the literature,^{23,24} we propose a second hypothetical approach to interpret this tendency: Higher aperiodic exponent values could reflect an inhibitory predominance $I \gg E$, whereas the lower values could correspond to an excitatory predominance $E \gg I$. Despite the presence of electrodes that do not fit the previously mentioned categorization in Figure 4, it is evident that in dyslaminated cortices, there is an imbalance between the two distributions. In contrast, in eulaminated cortices, the highest and lowest values are evenly distributed in terms of the number of electrodes, which may play a significant role in the dynamics during an activity disturbance. We also conduct a comparison between groups similar to what is shown in Figure 4F, focusing on aperiodic exponential values during the subsequent activities, Mg²⁺-Free and Recovery (Figure 5C). The most pronounced perturbations in signal distributions for both eulaminated and dyslaminated groups occur during two critical periods: The initial 15 min of 0 Mg²⁺ exposure and the recovery phase. Following an extended magnesium-free period, the distributions of both groups converge, particularly around the 30–45 min mark of perturbation.

The first 15 min represent a transient hyperexcitable state, where the network is maximally sensitive to the loss of Mg²⁺ blockade of NMDARs. This aligns with epileptiform activity models, where acute hyperexcitability, driven by unregulated Ca²⁺ influx and synchronous neuronal firing, precedes compensatory homeostatic adaptations. Prolonged absence of extracellular

Mg²⁺ further alters voltage dynamics, leading to hypersynchronized circuit activity, as reflected in the similarity of aperiodic exponent distributions between groups during this phase. However, the late recovery phase reveals a striking divergence between groups. The eulaminated (control) group demonstrates a robust return to baseline aperiodic activity, with increased density in the 1.0–1.2 range, indicative of effective homeostatic compensation. In contrast, the dyslaminated group fails to restore this peak, suggesting impaired recovery dynamics. This divergence likely stems from disrupted laminar connectivity in dyslaminated networks, which may hinder the coordination of inhibitory and excitatory adjustments required for stabilization.

These findings contribute to the critical role of structural integrity in network resilience. The 1.0–1.2 aperiodic range appears to represent a stable operational state for neuronal populations, potentially linked to optimal information processing or metabolic efficiency. The inability of dyslaminated networks to return to this range suggests persistent dysfunction, possibly due to altered inhibitory-excitatory balance or fragmented local circuitry.

Functional changes in typical bands during perturbation and recovery

To assess functional differences associated with perturbation induced by a magnesium-free medium and the subsequent recovery period, we analyzed a shared range of frequency fluctuations. This choice was motivated by established evidence that human seizure discharges often exhibit coupling between low and high frequencies.^{9,28} Our goal was to identify the frequency range showing the greatest variation in our recordings, drawing inspiration from Zhang, W. et al.,²⁹ who reported a consistent fluctuation band by analyzing temporal covariation in the power spectrum. We adapted their approach, considering that no specific frequency band has been universally defined for evoked epileptic activity. Our analysis revealed that the highest covariation occurred in the lower frequency range (0.5–30 Hz), and this effect was more pronounced in Dyslaminated cortices. We demonstrated this through the projection of the first two components of spectral covariation onto a weighted spectrogram and by projecting both modes onto a weighted power spectrum

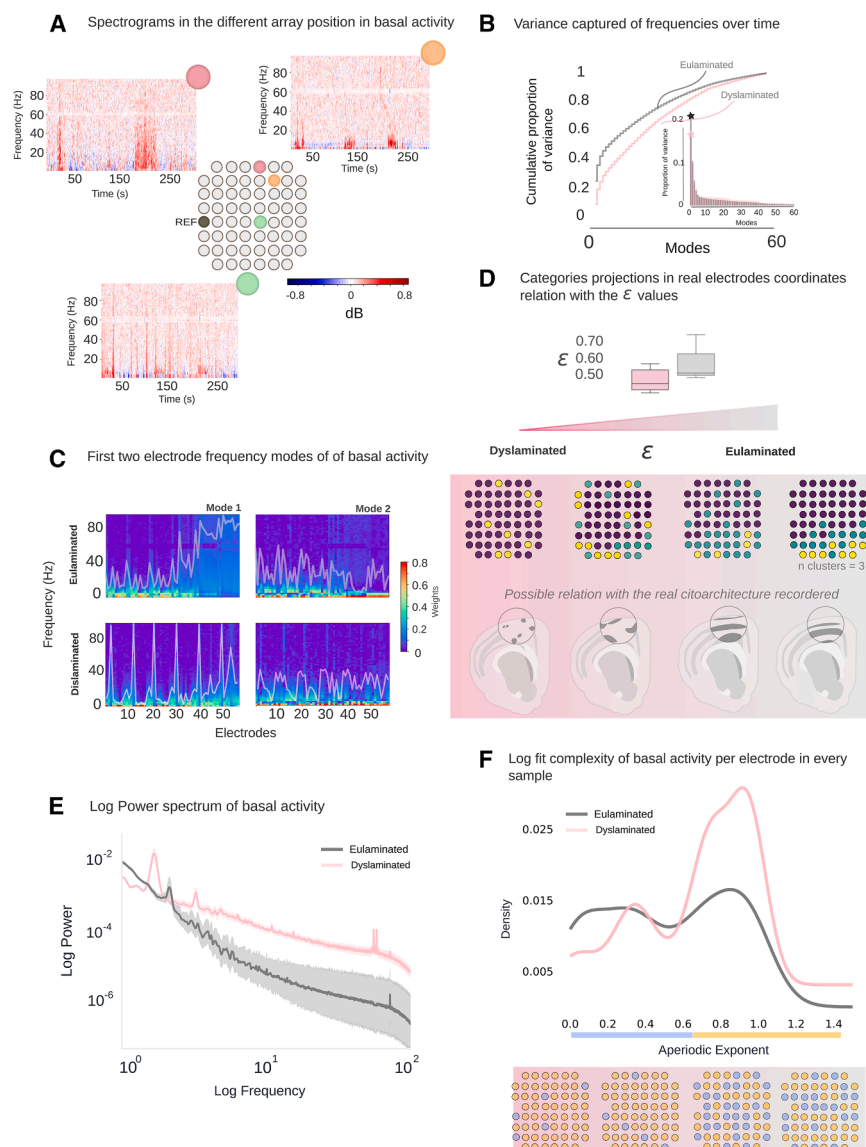


Figure 4. Classification of electrodes based on baseline activity to differentiate between Eulaminated and Dyslaminated cortices

Temporal frequency covariation.

(A) Normalized spectrograms showing baseline activity across various electrode positions. Example shown corresponds to an eulaminated extreme ($\epsilon = 0.75$), further spectrograms are shown in Figure S2.

(B) Variance captured from the frequency covariance in both groups (extreme examples, $\epsilon = 0.75$ and $\epsilon = 0.26$); eulaminated depicted in light gray, dyslaminated in light pink. Refer to Figure S3, to see all subject comparisons.

(C) Projection of the first two principal components (modes) into the comodulogram of frequency variation across all electrodes. The color scale indicates PCA weights; white lines illustrate mode fluctuations across electrodes.

(D) On top, ϵ values comparison from the layered disarray metric; all the recordings (Eulaminated = 3; Dyslaminated = 5). Significant difference ($p < 0.01$). In bottom, is depicted Electrode categories ($n = 3$) projected into real electrode positions and potentially linking to the level of cortical disorganization (hypothesis), at bottom. The extremes correspond to the same ϵ values as in previous panels. See complementary results in Figures S2 and S3.

(E) Logarithmic power spectrum for each electrode (extreme cases) along with aperiodic exponent values fitted across all records analyzed. Mean value in dark line, and the variance in shadow colors.

(F) Distribution of aperiodic exponent values from all the cases (Eulaminated = 3; Dyslaminated = 5).

(Figures 6A and 6B). Notably, covariance during baseline was consistently greater in Eulaminated cortices than in Dyslaminated ones (Figure 6C, upper panel). In contrast, during the Mg^{2+} -Free perturbation, cumulative variance between the two groups converged (Figure 6C, lower panel). This pattern is further illustrated in the representative logarithmic power spectra (Figure 6B, right), where baseline bandwidths maintain divergent decay slopes, while the Mg^{2+} -Free condition shows spectral convergence. This confirms our initial qualitative observations from Figure 3B, now filtered for the frequencies showing the highest covariance. To validate that the outcome of this bandwidth investigation preserves the ripple activity traits of the Mg^{2+} -Free model, we mapped the bandwidth threshold onto three electrodes chosen at random from different deepest MEA positions (upper, middle, and lower). As illustrated in Figure 6D, the observed activity changes persist under this constraint. To track the evolution of activity in relation to the pre-

viously defined levels of disorder, we analyze the transfer of information between the electrode categories identified, using the common band variation (Figure 7). Starting with baseline recordings (Figure 7A), we clarify that Dyslaminated samples conveyed lower information values compared to Eulaminated ones. Additionally, where the MEA shows lower ϵ values, the transfer of information becomes less structured and primarily occurs from the largest electrode category (purple dots) to the sparsity one (yellow dots).

It appears that the sparsity category transmits less information compared to the other, which may correspond to the hyper and hypoelectable regions previously suggested by Koh, H.⁶ but this time interpreted in terms of information content. However, as the ϵ values increased, a hierarchical pattern began to emerge in the flow of information,³⁰ progressing from deeper electrode categories to more superficial ones. This observation seems to align with the known hierarchical organization of the motor cortical circuit,³¹ particularly in the zone where we attempted to record Figure 2. We also investigated whether the ϵ values could reflect changes in information sharing along the cortical motor circuit. To this end, we assessed information dominance based on the depth position of the electrodes. Electrodes were grouped into

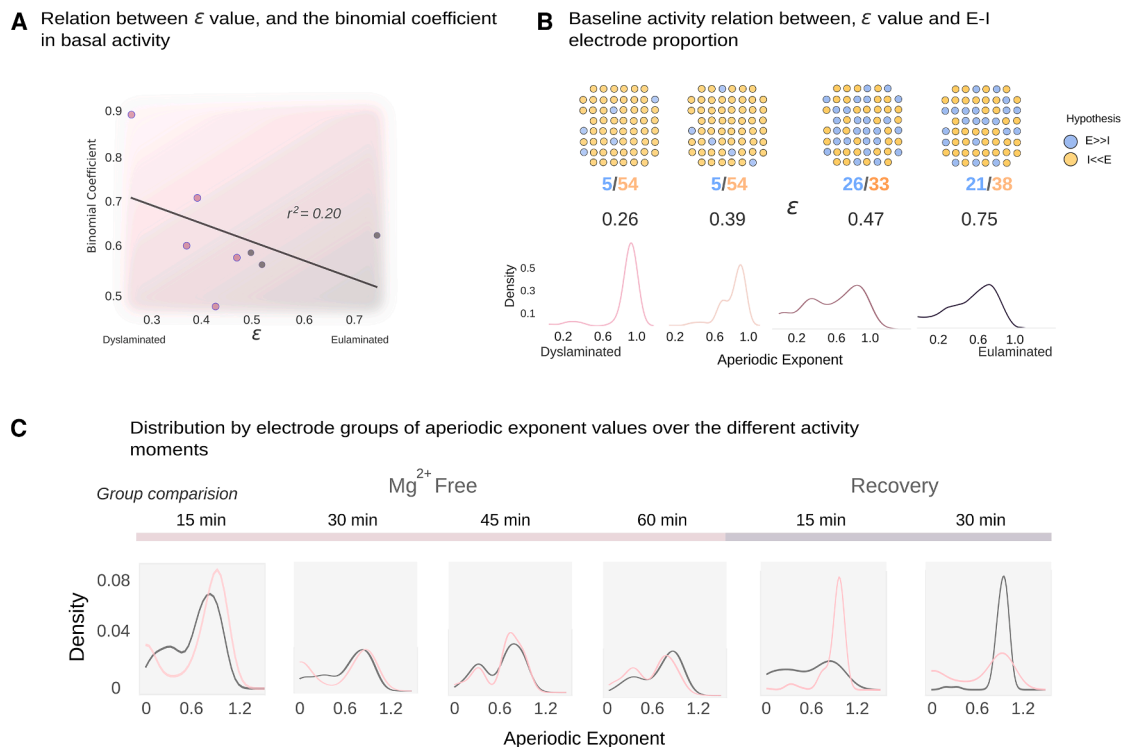


Figure 5. Relationship between the aperiodic exponent and the gradient of disorder in baseline activity, and its changes during perturbation and recovery

(A) Linear regression between the bimodal coefficient and the corresponding ϵ values ($r^2 = 0.20$, $p > 0.05$). Pink dots indicate dyslaminated arrays, whereas gray dots indicate eulaminated arrays.

(B) Electrode projections at different levels of structural disorder, with their associated ϵ values shown below each example. The proportions of aperiodic exponent values above and below 0.6 (Sensitivity: 0.71, Specificity: 0.53, and 95% CI for Accuracy: [0.612, 0.691]) are highlighted in orange and blue, respectively. Values below 0.6 are suggested as reflecting increased excitation ($E \gg I$), while values above 0.6 reflect increased inhibition ($I \gg E$). The specific spatial arrangement of the aperiodic exponent values for each example is displayed in the lower panel.

(C) Distribution of aperiodic exponent values across groups during consecutive phases of network activity: perturbation (Mg^{2+} -Free) and recovery.

three layers: upper (A), middle (B), and lower (C) (Figure 7C). Using the same procedure applied for transfer flow, we extracted all possible directional connections between these layers. Analysis of the directionality asymmetry³² allowed us to identify the dominant directions of information transfer between each partition. However, due to high variability in the results, the correlation remained low ($\rho = 0.24$, $p = 0.25$). Nevertheless, the eulaminated cortex exhibited consistent directional dominance in certain pathways, such as $A \rightarrow C$, $B \rightarrow C$, and $C \rightarrow A$, a stability that may reflect its normal functional organization.

We next asked which component of the signal carries the highest amount of information. Following the approach of Kayser, C.,³³ where they found that information amounts are distributed differently in the distinct components of an LFP signal, we analyzed the mean weighted spectrogram across the three activity paradigms (baseline/spontaneous activity, Mg^{2+} -Free and Recovery) and evaluated the information content of three signal components: phase, energy, and instantaneous frequency (Figure 7B, lower panel). These values reflect the average trend across all MEAs within the common LFP bandwidth, without distinguishing between levels of delamination, as the observed variability was insufficient for clear separation. Our results show that

phase carries the highest amount of information across conditions, while instantaneous frequency contributes the least. Furthermore, phase and energy follow similar trends over time. In Eulaminated cortices, both components begin with high information values during baseline activity but decrease during Mg^{2+} -Free perturbation and do not return to baseline levels during recovery. In contrast, Dyslaminated cortices also begin with high values, but their information content remains more stable over time. Additionally, the trends of phase and energy appear to evolve similarly over time in both conditions; on one hand, Eulaminated phases and energy begin with high information values, but as perturbation continues, they decrease and never return to their original levels, even during the recovery state. On the other hand, the Dyslaminated values also initiate at high information levels, but their trend shows minimal change over time. However, instant frequency shows the least informative values in both situations. Additionally, the pattern seen suggests that the Dyslaminated cortices seem to enhance their information content as time goes on and are likely to show higher values compared to the Eulaminated, during spontaneous activity and in the absence of Mg^{2+} , but again appear similar in the recovery phase, as with the other two components.

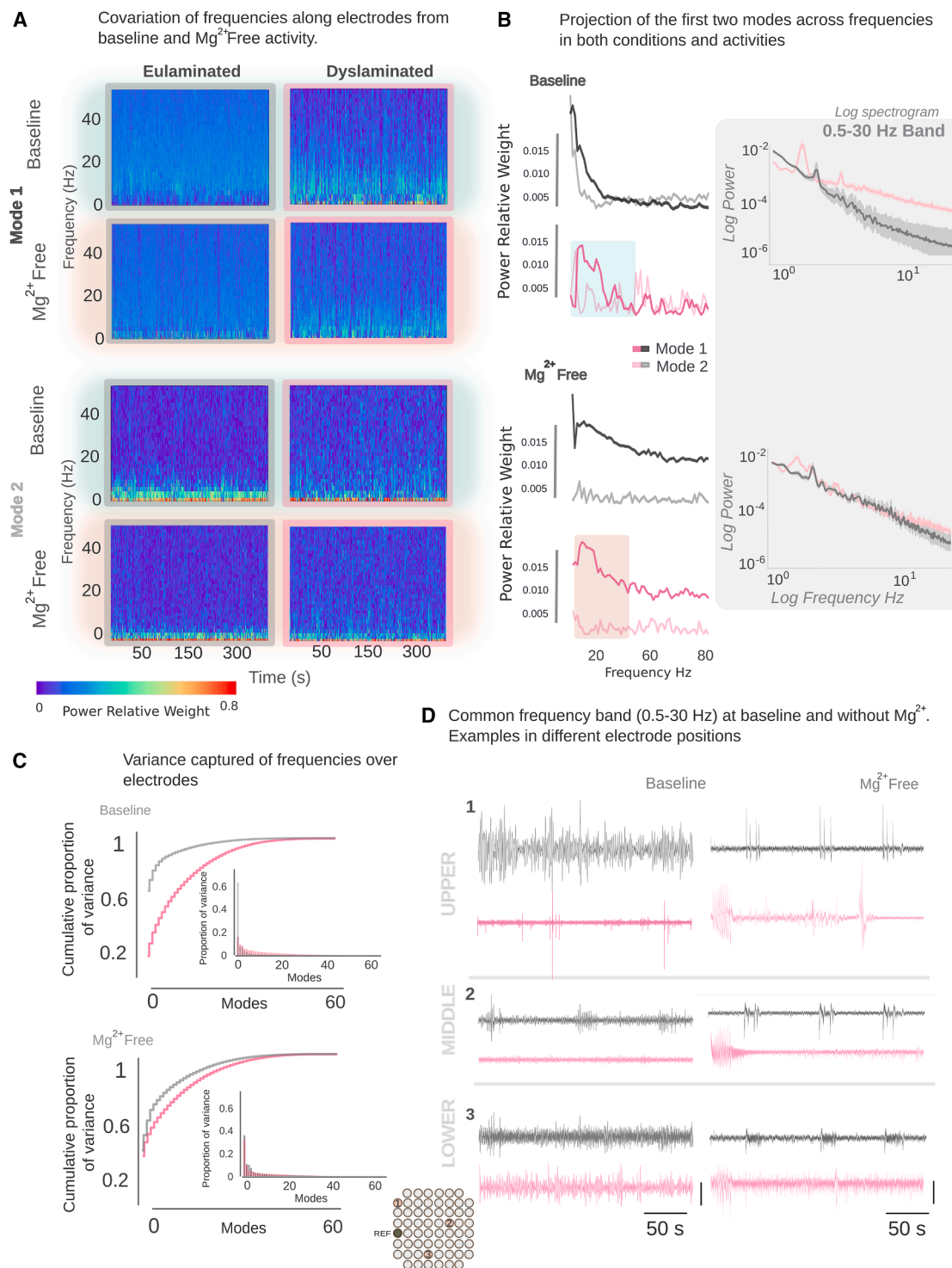


Figure 6. Common frequency band (0.5–30 Hz) selection from Dyslaminated cortices. Electrode frequency co-variation

(A) Spectrograms adjusted to highlight the first two modes during basal activity (depicted in green) and in Mg^{2+} -Free (shown in pale rose). Examples from extreme conditions. See Figure 2.

(legend continued on next page)

Temporal evolution of signal components

To better understand the temporal evolution of these components, we focused on the most informative component, the phase, and the least informative, the instantaneous frequency. Using mutual information computed between electrodes across the full shared LFP bandwidth and a sliding window approach, we quantified the dissimilarity between each instantaneous state and its subsequent state within each window (see Methods 10). Dimensionality reduction was then applied to these temporal dissimilarity matrices to track the evolution of each component across different activity paradigms (Figure 7D). The left panel presents a representative example from the extremes of the ϵ distribution. Each point corresponds to a time window, with color indicating temporal progression (red = early, blue = late). The ellipses represent the low-dimensional projections of the dynamics: red for frequency and blue for phase. For each multidimensional scaling (MDS) projection, an ellipse was fitted to quantify the spread of dynamics; larger ellipses indicate greater variability and a higher capacity to transform the underlying interaction dynamics. In the right panel, boxplots beneath these projections reveal that frequency dynamics were more temporally constrained, with slightly higher mean values observed in eulaminar cortices than in dyslaminar ones, except during the recovery phase. This observation aligns with the reduced information content shown in Figure 7B, supporting the interpretation that lower information corresponds to a diminished capacity for dynamic change. In contrast, phase dynamics exhibited greater variability in dyslaminar cortices across all activity paradigms, whereas eulaminar cortices showed increased variability only during the Mg^{2+} -Free condition. This highlights a key role for phase in modulating neural interactions under perturbed conditions. As with frequency, the average ellipse sizes for phase were generally larger in eulaminar cortices, except during recovery, where dyslaminar cortices showed increased variability that corresponded to elevated phase information content (Figure 7B). These findings offer valuable insights for future analytical decompositions and are consistent with previous studies emphasizing the importance of phase locking across frequency bands.^{10,33} Building upon the prior decomposition analysis, we re-examined the information transfer depicted in Figure 7A, this time focusing on distinct phases of activity while maintaining a consistent bandwidth. Figure 7E illustrates the same extreme cases from Figure 7A, now under Mg^{2+} -Free conditions (left) and during the recovery phase (right). In dyslaminar cortices, average information transfer shows a slight increase; notably, during the first 15 min of Mg^{2+} -Free exposure, the transfer flux remains aligned with baseline levels. However, this trend reverses over the subsequent 30 min. In contrast, eulaminar cortical activity displays a progressive decline in information transfer relative to spontaneous activity, with an

early disruption of hierarchical organization evident from the onset of Mg^{2+} -Free exposure. During recovery, the dyslaminar cortex rapidly re-establishes its original transfer pattern within the first 15 min, whereas the eulaminar cortex requires 30 min to partially restore hierarchical flow and fails to fully recover it. Collectively, these findings suggest that a certain degree of structural disorganization differentially recovers after activity perturbations. This disorganization appears to affect not only the excitatory-inhibitory balance but also the overall efficiency and stability of information transmission within the network. These insights may have broader implications for understanding how cortical circuits respond to and recover from functional disruptions, and how structural variability modulates their adaptive capacity.

DISCUSSION

Surgical resection remains the primary treatment for cortical dysplasia, which is strongly associated with pharmacoresistant epilepsy, particularly in pediatric populations.^{1,2} However, the functional variability inherent to cortical dysplasia often compromises the accurate delineation of the epileptogenic zone, underscoring the critical need to characterize the signal features that define and distinguish pathological cortical territories.³⁴ This clinical challenge motivated our investigation of the carmustine model across early stages of neurodevelopment¹¹. Although this model produces embryonic-onset, regionally diffuse, and heterogeneous malformations,¹⁵ This approach aligns with a central goal of translational neuroscience: improving the detection of pathological network states under resting-state conditions, with direct implications for pre-surgical planning. To deepen this analysis, we decomposed LFP spectrograms, recognizing that specific spectral components may reflect underlying cognitive and pathological states. In line with EEG based approaches^{9,35,36} this analysis revealed functional signatures potentially tied to local cytoarchitecture. Notably, we observed patterns consistent with previously described neuron-type-specific signal features.³⁷ By mapping activity classifications using our proposed metric ϵ , we characterized spatial heterogeneity in functional groups, distinguishing disorganized (Dyslaminar) from more structured (Eulaminar) cortical regions. We further explored the aperiodic exponent of LFP signals to assess its relationship with these functional classifications. Interestingly, a bimodal distribution emerged, with higher divergence in Dyslaminar regions and greater uniformity in Eulaminar areas. This dual pattern may reflect distinct (E/I) regimes, as previously had been suggested,^{38,39} echoing previous findings from both cognitive and epileptic contexts.^{7,8,23,24,40} Under Mg^{2+} -Free perturbation, this distinction faded, suggesting that strong ionic shifts may transiently override intrinsic network heterogeneity. After recovery, the absence of the bimodal pattern implies a

(B) The power spectrum weighted according to the first two modes averaged across all samples (eulaminar = 4; dyslaminar = 5). Panel right indicates the frequency range where power was most prominent. The dark line represents the mean log spectrum, while the shaded region shows the variance across spectra. (C) Variance distributions captured in both conditions (baseline and in Mg^{2+} -Free). Light pink corresponds to dyslaminar cortices and light gray to eulaminar. (D) Examples of baseline and Mg^{2+} -Free activity after filtering by the common frequency band, shown across three MEA depths (lower, middle, upper). Examples in panels C and D correspond to the extreme conditions. See further details in Figure S4.

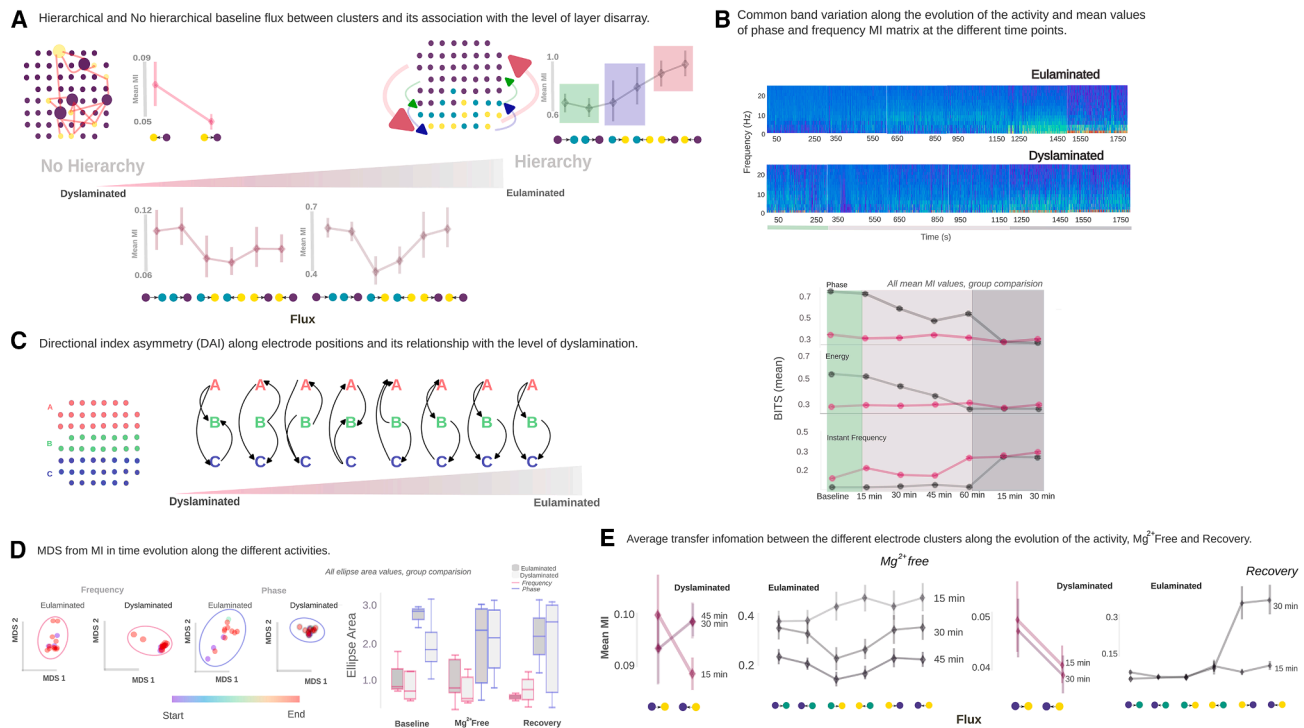


Figure 7. Global dynamical evolution of the common frequency band and its relationship with information flow across electrode categories (A) Baseline information flux for extreme dyslaminated and eulaminated samples, alongside two intermediate cases ($\epsilon = 0.26$ and $\epsilon = 0.75$, respectively). Each scheme illustrates the presence or absence of hierarchy in information flow (left: no hierarchy; right: hierarchy). Bottom plots show the mean information carried across directions; points represent the mean, and error bars denote the 95% confidence interval estimated by bootstrapping. (B) Average spectrogram within the shared frequency band defined in Figure 3, representing total activity captured across conditions (Baseline in light green, Mg²⁺-Free in pale pink, Recovery in pale violet). Bottom: mean information (in bits) over time for each signal component within the common band—phase, energy, and instantaneous frequency. (C) Relationship between ϵ values and electrode depth in information flow. The left panel shows the MEA array with electrodes partitioned into upper (red—A), middle (green—B), and lower (blue—C) layers. The right panel depicts a schematic of cortical laminar index (DAI) dominance for each sample, ranging from dyslaminated to eulaminated cortex. The ordering of ϵ values corresponds to Figure S3. No statistically significant relationship was observed ($p > 0.05$). (D) Multidimensional scaling (MDS) analysis of dissimilarity in mutual information matrices for each activity condition, with ellipses fitted around each point to represent the spread of dynamics in low-dimensional space (right: temporal evolution of frequency; left: temporal evolution of phase). Bottom: ellipse area values across all recordings and activity conditions. (E) Information flux during the Mg²⁺-Free and Recovery phases for the same extreme dyslaminated and eulaminated samples shown in panel A. Points and error bars follow the same convention as panel A. Statistical comparisons between additional samples are shown in Figure S5.

homogenization of network activity, masking the functional distinctions evident during spontaneous states, distinctions likely rooted in cytoarchitectural organization.

These observations, together with the differences in inferred baseline information transmission between electrode categories, point to a possible alteration of the E/I balance at baseline.⁴¹ Furthermore, the extent of cortical delamination may play a role in shaping signal propagation, especially when the network is challenged by external perturbations such as Mg²⁺-Free medium.

In response to external perturbations, our analysis revealed that the most informative features for electrode categorization, signal aperiodicity, and inter-category transfer entropy consistently emerged from spontaneous activity. This highlights the importance of intrinsic dynamics in uncovering structural-functional relationships and supports the broader applicability of our methodology. Thus, we propose that the key signal features in both healthy and

diseased states could be assessed using this approach. This methodology may be adapted to study functional inhomogeneities across various experimental paradigms, including healthy brain states and different neurological conditions, potentially providing a simplified method for pre-surgical evaluation.

Limitations of the study

This study presents several limitations inherent to the use of an *ex vivo* model of cortical malformation. A primary constraint is the inability to directly validate the functional findings with the histological analysis of the exact recorded regions. This is due to the use of multielectrode array (MEA) recordings in 400 μ m-thick cortical slices, which often sustain substantial structural damage after prolonged recording sessions, rendering subsequent histological processing unfeasible. Nevertheless, our histological validation was performed using tissue from animals in the same cohort. Thus, all links between ϵ and cytoarchitectural

disorder should be interpreted as hypothesis-driven and population-level rather than slice-specific. However, it should be noted that the carmustine model is well characterized at the cytological level, and the morphological features observed in our slices were consistent with those extensively described in the literature, including disrupted cortical layering, heterotopic neurons, and altered cellular organization that closely mirror the cytoarchitectural hallmarks of FCD. While the model effectively replicates these key structural features, it does not fully capture the complexity and heterogeneity observed in human cases. Therefore, future studies applying similar analytical frameworks to human tissue, particularly samples obtained from epilepsy surgeries, will be essential for strengthening translational relevance and assessing the generalization of the present findings.

RESOURCE AVAILABILITY

Lead contact

Request for further information and resources or reagents should be directed to and fulfilled by the lead contact, Roman Rossi Pool romanr@ifc.unam.mx.

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data: MEA recordings are publicly accessible via Zenodo <https://doi.org/10.5281/zenodo.15592455>.
- Code: The source code and all analysis scripts are openly available on, <https://doi.org/10.5281/zenodo.15592455>.
- Additional information: Further information is available from the [lead contact](#) upon request.

ACKNOWLEDGMENTS

We extend our sincere gratitude to the members of the Rossi-Pool Laboratory for their dedication and contributions throughout the development of this project. We are also grateful to Sergio Parra and Jerónimo Zizumbo for their valuable suggestions and feedback during the early stages of this manuscript. Special thanks go to Alessio Franci and Lucas Bayones for their insightful comments during the editing process. We acknowledge Leopoldo Gonzalez Santos for his technical support with cluster computing, Benito Ordaz for his technical support maintaining brain slicing and recording devices, as well as the Biotherium Facility at the Institute of Neurobiology and Ericka de los Rios and Nydia Hernández Rios for their support in histological evaluation and support in capturing the images. We appreciate the support of the PhD Program in Biomedical Sciences at the National Autonomous University of Mexico (UNAM). This work was financially supported by UNAM-DGAPA PAPIIT (IN208725 FPO, IN204720 LC, IN203825 RRP, IA200621 HLM), PASPA (FPO), CONACYT (FC1782) and SECIHTI (CBF-2025-I-2054 RRP). Ana Aquiles received scholarship support from CONACYT (no. 756903).

AUTHOR CONTRIBUTIONS

A.A., R.R.P., L.C., L.M.H., and P.O.F. contributed to the conception of the study and offered experimental method guidance. P.V.L. performed the experimental procedure of MEA recordings. M.R. performed the histological procedure and image acquisition. A.A. and R.R.P. designed the algorithm, performed formal analysis and visualization, and drafted the manuscript. L.C., P.O.F., and P.V.L. provided valuable suggestions for the experiments and manuscript. All authors contributed to the review and editing of the manuscript.

DECLARATION OF INTERESTS

Non declared.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work the authors used Grammarly in order to improve the syntax, grammar, and flow of selected sections of this manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS**
 - Animals
- **METHOD DETAILS**
 - Slice preparation
 - Electrophysiological recordings
 - Immunofluorescence
 - Preprocessing of electrophysiological data
 - LFP spectrograms
 - Dimensionality reduction and electrode clustering
 - Level of disarrangement metric (ϵ)
 - Common band determination
 - Aperiodic exponent fitting and bimodality
 - Transfer entropy along electrode clusters
 - Electrode depth segmentation of the MEA
 - Multi-set directionality computation
 - Directionality asymmetry index
 - MI of LFP components (magnitude, phase, and instantaneous frequency) during time evolution
 - Pairwise dissimilarity evaluation and multidimensional scaling
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
 - Quality control
 - Aperiodic exponent thresholding and bimodality
 - Multidimensional scaling and condition comparison
 - Group comparison of the disarrangement metric (ϵ)
 - Permutation test for correlation between ϵ and the directionality asymmetry index
 - Permutation-based significance testing of mutual information
 - Comparison of directional information flow

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2026.115942>.

Received: July 17, 2025

Revised: October 22, 2025

Accepted: April 27, 2026

Published: April 30, 2026

REFERENCES

1. Cohen, N.T., Chang, P., You, X., Zhang, A., Havens, K.A., Oluigbo, C.O., Whitehead, M.T., Gholipour, T., and Gaillard, W.D. (2022). Prevalence and Risk Factors for Pharmacoresistance in Children With Focal Cortical Dysplasia-Related Epilepsy. *Neurology* 99, e2006–e2013. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9651467/>.
2. Diaz, R.J., Sherman, E.M.S., and Hader, W.J. (2008). Surgical treatment of intractable epilepsy associated with focal cortical dysplasia. *Neurosurg. Focus* 25, E6. <https://doi.org/10.3171/FOC/2008/25/9/E6>.
3. Maynard, L.M., Leach, J.L., Horn, P.S., Spaeth, C.G., Mangano, F.T., Holland, K.D., Miles, L., Faist, R., and Greiner, H.M. (2017). Epilepsy prevalence and severity predictors in MRI-identified focal cortical dysplasia.

- Epilepsy Res. 132, 41–49. <https://doi.org/10.1016/j.eplepsyres.2017.03.001>. <https://www.sciencedirect.com/science/article/pii/S0920121116302790>.
4. Kim, S.H., and Choi, J. (2023). Pathological Classification of Focal Cortical Dysplasia (FCD) : Personal Comments for Well Understanding FCD Classification. *J. Korean Neurosurg. Soc.* 62, 288–295. <https://doi.org/10.3340/jkns.2019.0025>. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6514319/>.
5. Abdijadid, S., Mathern, G.W., Levine, M.S., and Cepeda, C. (2015). Basic Mechanisms of Epileptogenesis in Pediatric Cortical Dysplasia. *CNS Neurosci. Ther.* 21, 92–103. <https://doi.org/10.1111/cns.12345>.
6. Koh, H.Y., Jang, J., Ju, S.H., Kim, R., Cho, G.B., Kim, D.S., Sohn, J.W., Paik, S.B., and Lee, J.H. (2021). Non-Cell Autonomous Epileptogenesis in Focal Cortical Dysplasia. *Ann. Neurol.* 90, 285–299. <https://doi.org/10.1002/ana.26149>.
7. Xie, K., Royer, J., Rodriguez-Cruces, R., Horwood, L., Ngo, A., Arafat, T., Auer, H., Sahlas, E., Chen, J., Zhou, Y., et al. (2025). Temporal Lobe Epilepsy Perturbs the Brain-Wide Excitation-Inhibition Balance: Associations with Microcircuit Organization, Clinical Parameters, and Cognitive Dysfunction. *Adv. Sci.* 12, e2406835. <https://doi.org/10.1002/advsc.202406835>.
8. Donoghue, T., Hammonds, R., Lybrand, E., Washcke, L., Gao, R., and Voytek, B. (2024). Evaluating and Comparing Measures of Aperiodic Neural Activity. Preprint at bioRxiv. <https://doi.org/10.1101/2024.09.15.613114>. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11419150/>.
9. Ricci, L., Tamilia, E., Mercier, M., Pepi, C., Carfi-Pavia, G., De Benedictis, A., Assenza, G., Di Lazzaro, V., Vigeveno, F., Specchio, N., et al. (2023). Phase-amplitude coupling between low- and high-frequency activities as preoperative biomarker of focal cortical dysplasia subtypes. *Clin. Neurophysiol.* 150, 40–48. <https://doi.org/10.1016/j.clinph.2023.03.006>. <https://www.sciencedirect.com/science/article/pii/S138824572300233X>.
10. Schroeder, G.M., Diehl, B., Chowdhury, F.A., Duncan, J.S., de Tisi, J., Trevelyan, A.J., Forsyth, R., Jackson, A., Taylor, P.N., and Wang, Y. (2020). Seizure pathways change on circadian and slower timescales in individual patients with focal epilepsy. *Proc. Natl. Acad. Sci.* 117, 11048–11058. <https://doi.org/10.1073/pnas.1922084117>.
11. Benardete, E.A., and Kriegstein, A.R. (2002). Increased Excitability and Decreased Sensitivity to GABA in an Animal Model of Dysplastic Cortex. *Epilepsia* 43, 970–982. <https://doi.org/10.1046/j.1528-1157.2002.40901.x>.
12. Moroni, R.F., Cipelletti, B., Inverardi, F., Regondi, M.C., Spreafico, R., and Frassoni, C. (2011). Development of cortical malformations in BCNU-treated rat, model of cortical dysplasia. *Neuroscience* 175, 380–393. <https://doi.org/10.1016/j.neuroscience.2010.11.061>. <https://www.sciencedirect.com/science/article/pii/S0306452210015526>.
13. Moroni, R.F., Inverardi, F., Regondi, M.C., Watakabe, A., Yamamori, T., Spreafico, R., and Frassoni, C. (2009). Expression of layer-specific markers in the adult neocortex of BCNU-Treated rat, a model of cortical dysplasia. *Neuroscience* 159, 682–691. <https://doi.org/10.1016/j.neuroscience.2008.12.064>. <https://www.sciencedirect.com/science/article/pii/S0306452208018575>.
14. Moroni, R.F., Inverardi, F., Regondi, M.C., Panzica, F., Spreafico, R., and Frassoni, C. (2008). Altered spatial distribution of PV-cortical cells and dysmorphic neurons in the somatosensory cortex of BCNU-treated rat model of cortical dysplasia. *Epilepsia* 49, 872–887. <https://doi.org/10.1111/j.1528-1167.2007.01440.x>.
15. Aquiles, A., Fiordelisio, T., Luna-Munguía, H., and Concha, L. (2023). Altered functional connectivity and network excitability in a model of cortical dysplasia. *Sci. Rep.* 13, 12335. <https://doi.org/10.1038/s41598-023-38717-2>.
16. Ortega-Fimbres, O., Ríos-Carrillo, R., Gaspar-Martínez, E., Ruiz-Acosta, P., Regalado, M., Luna-Munguía, H., and Ramírez-Manzanares, A. (2026). Analysis of cortical dysplasias using b-tensor encoding diffusion MRI in an animal model. *PLoS One* 27, e0346132. <https://doi.org/10.1371/journal.pone.0346132>.
17. Villaseñor, P.J., Cortés-Servín, D., Pérez-Moriél, A., Aquiles, A., Luna-Munguía, H., Ramírez-Manzanares, A., Coronado-Leija, R., Larriva-Sahd, J., and Concha, L. (2023). Multi-tensor diffusion abnormalities of gray matter in an animal model of cortical dysplasia. *Front. Neurol.* 14, 1124282. <https://doi.org/10.3389/fneur.2023.1124282>.
18. Coan, E.J., and Collingridge, G.L. (1985). Magnesium ions block an N-methyl-D-aspartate receptor-mediated component of synaptic transmission in rat hippocampus. *Neurosci. Lett.* 53, 21–26. [https://doi.org/10.1016/0304-3940\(85\)90091-6](https://doi.org/10.1016/0304-3940(85)90091-6).
19. Avoli, M., Drapeau, C., Louvel, J., Pumain, R., Olivier, A., and Villemure, J.G. (1991). Epileptiform activity induced by low extracellular magnesium in the human cortex maintained in vitro. *Ann. Neurol.* 30, 589–596. <https://doi.org/10.1002/ana.410300412>.
20. Jones, R.S., and Heinemann, U. (1988). Synaptic and intrinsic responses of medial entorhinal cortical cells in normal and magnesium-free medium in vitro. *J. Neurophysiol.* 59, 1476–1496. <https://doi.org/10.1152/jn.1988.59.5.1476>.
21. Igelström, K.M., Shirley, C.H., and Heyward, P.M. (2011). Low-magnesium medium induces epileptiform activity in mouse olfactory bulb slices. *J. Neurophysiol.* 106, 2593–2605. <https://doi.org/10.1152/jn.00601.2011>.
22. Isaev, D., Ivanchick, G., Khmyz, V., Isaeva, E., Savrasova, A., Krishtal, O., Holmes, G.L., and Maximyuk, O. (2012). Surface charge impact in low-magnesium model of seizure in rat hippocampus. *J. Neurophysiol.* 107, 417–423. <https://doi.org/10.1152/jn.00574.2011>. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3349697/>.
23. Brake, N., Duc, F., Rokos, A., Arseneau, F., Shahiri, S., Khadra, A., and Plourde, G. (2024). A neurophysiological basis for aperiodic EEG and the background spectral trend. *Nat. Commun.* 15, 1514. <https://doi.org/10.1038/s41467-024-45922-8>.
24. Donoghue, T., Haller, M., Peterson, E.J., Varma, P., Sebastian, P., Gao, R., Noto, T., Lara, A.H., Wallis, J.D., Knight, R.T., et al. (2020). Parameterizing neural power spectra into periodic and aperiodic components. *Nat. Neurosci.* 23, 1655–1665. <https://doi.org/10.1038/s41593-020-00744-x>. <https://www.nature.com/articles/s41593-020-00744-x>.
25. Avoli, M., Drapeau, C., and Kostopoulos, G. (1988). Changes in Synaptic Transmission Evoked in the “In Vitro” Hippocampal Slice by a Brief Decrease of [Mg⁺⁺]₀: A Correlate for Long-Term Potentiation? In *Synaptic Plasticity in the Hippocampus*, Haas HL, and Buzsáki G., eds. (Springer). https://link.springer.com/chapter/10.1007/978-3-642-73202-7_3.
26. Tancredi, V., Hwa, G.G.C., Zona, C., Brancati, A., and Avoli, M. (1990). Low magnesium epileptogenesis in the rat hippocampal slice: electrophysiological and pharmacological features. *Brain Res.* 511, 280–290. <https://www.sciencedirect.com/science/article/pii/0006899390901739>.
27. Dura-Bernal, S., Neymotin, S.A., Suter, B.A., Dacre, J., Moreira, J.V.S., Urdapilleta, E., Schiemann, J., Duguid, I., Shepherd, G.M.G., and Lytton, W.W. (2023). Multiscale model of primary motor cortex circuits predicts *in vivo* cell-type-specific, behavioral state-dependent dynamics. *Cell Rep.* 42, 112574. <https://doi.org/10.1016/j.celrep.2023.112574>. <https://www.sciencedirect.com/science/article/pii/S2211124723005855>.
28. Kuroda, N., Sonoda, M., Miyakoshi, M., Nariai, H., Jeong, J.W., Motoi, H., Luat, A.F., Sood, S., and Asano, E. (2021). Objective interictal electrophysiology biomarkers optimize prediction of epilepsy surgery outcome. *Brain Commun.* 3, fcab042. <https://doi.org/10.1093/braincomms/fcab042>.
29. Zhang, W., and Yartsev, M.M. (2019). Correlated Neural Activity across the Brains of Socially Interacting Bats. *Cell* 178, 413–428.e22. <https://doi.org/10.1016/j.cell.2019.05.023>. <https://www.sciencedirect.com/science/article/pii/S0092867419305513>.
30. Tauste Campo, A., Zainos, A., Vázquez, Y., Segarra, R., Álvarez, J., Deco, G., Díaz, J.A., Parra, S., Romo, R., and Rossi-Pool, R. (2024). Thalamo-cortical interactions shape hierarchical neural variability during stimulus perception. *iScience* 27, 110065. <https://doi.org/10.1016/j.isci.2024.110065>.
31. Hooks, B.M., Mao, T., Gutnisky, D.A., Yamawaki, N., Svoboda, K., and Shepherd, G.M. (2013). Organization of Cortical and Thalamic Input to

- Pyramidal Neurons in Mouse Motor Cortex. *J. Neurosci.* 33, 748–760. <https://doi.org/10.1523/JNEUROSCI.4338-12.2013>. <https://www.jneurosci.org/content/33/2/748>.
32. Jelfs, B., and Chan, R.H.M. (2017). Directionality indices: Testing information transfer with surrogate correction. *Phys. Rev. E* 96, 052220. <https://doi.org/10.1103/PhysRevE.96.052220>.
33. Kayser, C., Montemurro, M.A., Logothetis, N.K., and Panzeri, S. (2009). Spike-phase coding boosts and stabilizes information carried by spatial and temporal spike patterns. *Neuron* 61, 597–608. <https://doi.org/10.1016/j.neuron.2009.01.008>.
34. Janca, R., Jezdik, P., Ebel, M., Kalina, A., Kudr, M., Jahodova, A., Krysl, D., Mackova, K., Straka, B., Marusic, P., et al. (2023). Distinct patterns of interictal intracranial EEG in focal cortical dysplasia type I and II. *Clin. Neurophysiol.* 151, 10–17. <https://doi.org/10.1016/j.clinph.2023.03.360>.
35. Varotto, G., Tassi, L., Franceschetti, S., Spreafico, R., and Panzica, F. (2012). Epileptogenic networks of type II focal cortical dysplasia: A stereo-EEG study. *Neuroimage* 61, 591–598. <https://doi.org/10.1016/j.neuroimage.2012.03.090>. <https://www.sciencedirect.com/science/article/pii/S1053811912003783>.
36. Rampp, S., Rössler, K., Hamer, H., Illek, M., Buchfelder, M., Doerfler, A., Pieper, T., Hartlieb, T., Kudernatsch, M., Koelble, K., et al. (2021). Dysmorphic neurons as cellular source for phase-amplitude coupling in Focal Cortical Dysplasia Type II. *Clin. Neurophysiol.* 132, 782–792. <https://doi.org/10.1016/j.clinph.2021.01.004>. <https://www.sciencedirect.com/science/article/pii/S1388245721000109>.
37. Dura-Bernal, S., Herrera, B., Lupascu, C., Marsh, B.M., Gandolfi, D., Marasco, A., Neymotin, S., Romani, A., Solinas, S., Bazhenov, M., et al. (2024). Large-Scale Mechanistic Models of Brain Circuits with Biophysically and Morphologically Detailed Neurons. *J. Neurosci.* 44, e1236242024. <https://doi.org/10.1523/JNEUROSCI.1236-24.2024>.
38. Gao, R., van den Brink, R.L., Pfeffer, T., and Voytek, B. (2020). Neuronal timescales are functionally dynamic and shaped by cortical microarchitecture. *eLife* 9, e61277. <https://doi.org/10.7554/eLife.61277>.
39. Pani, S.M., Saba, L., and Fraschini, M. (2022). Clinical applications of EEG power spectra aperiodic component analysis: A mini-review. *Clin. Neurophysiol.* 143, 1–13. <https://doi.org/10.1016/j.clinph.2022.08.010>. <https://www.sciencedirect.com/science/article/pii/S138824572200863X>.
40. Donoghue, T. (2025). A historical overview of the study of aperiodic neural activity. https://osf.io/zrvxa_v1.
41. Rossi Pool, R., and Mato, G. (2011). Inferring single neuron properties in conductance-based balanced networks. *Front. Comput. Neurosci.* 5, 41. <https://doi.org/10.3389/fncom.2011.00041>.
42. Méndez-Salcido, F.A., Torres-Flores, M.I., Ordaz, B., and Peña-Ortega, F. (2022). Abnormal innate and learned behavior induced by neuron–microglia miscommunication is related to CA3 reconfiguration. *Glia* 70, 1630–1651. <https://doi.org/10.1002/glia.24185>.
43. Laura, P.-V., Felipe, M.-S., Jonathan-Julio, L.-H., de Lafuente, V., and Fernando, P.-O. (2025). Combined Effects of Intermittent Hypoxia and Amyloid Beta on Hippocampal Activity, Its Cholinergic Modulation, and Memory. *Hippocampus* 35, e70017. <https://doi.org/10.1002/hipo.70017>. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC12152712/>.
44. Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., et al. (2011). Scikit-learn: Machine learning in Python. *J. Mach. Learn. Res.* 12, 2825–2830. http://www.jmlr.org/papers/volume12/pedregosa11a/pedregosa11a.pdf?source=post_page.
45. Miller, K.J., Sorensen, L.B., Ojemann, J.G., and Nijs, M.d. (2009). Power-Law Scaling in the Brain Surface Electric Potential. *PLoS Comput. Biol.* 5, e1000609. <https://doi.org/10.1371/journal.pcbi.1000609>. <https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1000609>.
46. Virtanen, P., Gommers, R., Oliphant, T.E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., et al. (2020). SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nat. Methods* 17, 261–272. <https://doi.org/10.1038/s41592-019-0686-2>. <https://www.nature.com/articles/s41592-019-0686-2>.
47. Freeman, J.B., and Dale, R. (2013). Assessing bimodality to detect the presence of a dual cognitive process. *Behav. Res. Methods* 45, 83–97. <https://doi.org/10.3758/s13428-012-0225-x>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
Domestic Norwegian rat <i>Rattus norvegicus</i> (Sprague-Dawley)	Wild	NA
Chemicals and antibodies		
Carmustine	Sigma-Aldrich	C0400
Anti-NeuN	Abcam	ab104224
Alexa 488	Invitrogen	RRID:AB_10711040
Data availability		
Rat MEA recordings	This paper	zenodo.org/records/15592455
Software and algorithms		
MC Rack software	Multi Channel Systems (MCS GmbH)	McsPyDataTools
Python 3.10	Python Software Foundation	python.org
CorticalDelam-MEA	This paper	zenodo.org/records/15592455

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animals

Protocols followed in this study were approved by the ethics committee of the Institute of Neurobiology, UNAM (Protocol 111-A) and carried out in accordance with federal regulatory laws for animal experimentation (NOM-062-ZOO-1999). Pregnant Sprague-Dawley rats were intraperitoneally injected with BCNU following as previously reported methods,^{11,15} with 20 mg/kg diluted in 5% sucrose) on gestational day E15, for the Dyslaminated group, $n = 3$. For the Eulaminated group, $n = 2$ dams were injected with saline solution, at the same gestational day. All animals were kept in a room with a 12-h light/dark cycle and had unlimited access to food and water. For the Eulaminated group, five offspring from the dams were used for electrical recording $n = 5$ and three for histological analysis $n = 3$. For the Dyslaminated group $n = 7$ animals were employed for electrophysiology and $n = 5$ for histological validation.

METHOD DETAILS

Slice preparation

To conduct Multielectrode Array (MEA) recordings, we obtained 400 μm -thick coronal slices of the primary motor cortex (M1; bregma +0.20 mm) from male rats. Rats were anesthetized with pentobarbital (60 mg/kg) and transcardially perfused with a cold sucrose-based artificial cerebrospinal fluid (aCSF) containing the following (in mM): 3 KCl, 2.5 MgCl_2 , 25 NaHCO_3 , 238 sucrose, and 30 D-glucose, as also employed previously.^{42,43} The solution was maintained at pH 7.4 with constant carbogen bubbling. The brain was then removed and placed in an ice-cold carbogenated solution (95% O_2 /5% CO_2). Coronal slices were cut using a vibratome (HM-650-V, Thermo Fisher, or VT1000S, Leica) and transferred to a slice recovery and maintenance solution (in mM): 119 NaCl, 3 KCl, 1.5 CaCl_2 , 1 MgCl_2 , 25 NaHCO_3 , and 30 D-glucose. This solution was maintained at pH 7.4, oxygenated, and incubated at 34 °C for at least 90 min before use. Each recording was performed using slices from a different animal. Mg^{2+} -Free solution composition was the following (in mM): 120 NaCl, 5 KCl, 1.5 CaCl_2 , 26 NaHCO_3 , and 30 D-glucose.

Electrophysiological recordings

Recordings were obtained using a 60-electrode MEA (8 × 8 grid, 60pMEA200/30iR-Ti, Multichannel Systems), perfused from above and below with circulating aCSF at 34 °C, bubbled with carbogen. Electrodes targeted the primary motor cortex, verified via bregma distance and stereotaxic microscope. Negative pressure was applied to ensure adhesion of the slice. Recordings were acquired at 25 kHz for 15 min. Baseline activity was recorded, followed by Mg^{2+} -Free aCSF recordings (3 samples of 15 min), and then recovery activity, with normal aCSF (2 samples of 15 min).

Immunofluorescence

Consecutive coronal brain slices obtained from animals used in this study were collected and processed to evaluate neuronal morphology in both experimental groups: Eulaminated ($n = 1$) and Dyslaminated ($n = 3$). Brain slices were placed in a cryoprotective

solution and subsequently embedded in a cryomatrix. Tissues were rapidly frozen using absolute ethanol cooled with liquid nitrogen and stored at -20°C until sectioning.

Coronal sections of $20\ \mu\text{m}$ thickness were cut using a cryostat. Sections were preserved in $1\times$ PBS (w/v) and then washed five times for 10 min each in $1\times$ PBS containing 0.3% (w/v) Tween 20 (Sigma-Aldrich). Blocking was performed for 45 min at room temperature using a blocking solution composed of $1\times$ PBS, 0.3% (v/v) Triton X-100, and 2% (w/v) bovine serum albumin (BSA).

Following blocking, sections were incubated overnight at 4°C with the primary antibody NeuN (ab104224) diluted 1:350 in blocking solution. After washing, sections were incubated with fluorescently conjugated secondary Alexa 488 (Invitrogen a32723), a goat anti-mouse antibody, diluted 1:500 in blocking solution for 4 h at 4°C . Sections were then coverslipped with microscope cover glass.

Immunofluorescence imaging was performed using a laser scanning confocal microscope (Zeiss LSM 780 DUO).

Preprocessing of electrophysiological data

Raw MEA recordings were decompressed and extracted from MC Rack software using McsPyDataTools. We then applied a low-pass filter using a 5th-order Butterworth filter with a cutoff frequency of 100 Hz. Electrode traces were downsampled to 1 kHz. Power-line noise was eliminated using a notch filter with a quality factor of 90. We then reorganized the index of each electrode to recreate the original architecture of the 60-electrode MEA (8×8 grid), and kept the reference electrode off for all further analyses. Quality control was performed by comparison of the logarithmic power spectrum and revisited during dimensionality reduction of the LFP. Sample sizes after quality control were: Eulaminated $n = 3$; Dyslaminated $n = 5$.

LFP spectrograms

Spectrograms were computed using 1-s sliding windows with 75% overlap. Power spectra over 0.5–100 Hz were computed using 6 tapers and multi-taper decomposition. The number of frequency points was reduced to 60, and power was normalized using baseline mean power.

Dimensionality reduction and electrode clustering

To characterize how the spectrogram varies along electrodes for different frequencies f as a function of time t , we performed PCA on the spectrograms computed as described above. The number of significant dimensions spans up to 60 bins (0.5–100 Hz). PCA was performed from the covariance matrix of every electrode spectrogram over the time windows t and frequencies f as follows:

$$C_{ij} = \frac{1}{TF} \sum_{t=1}^T \sum_{f=1}^F (\text{spec}_{i,f}(t) - \overline{\text{spec}}_i) (\text{spec}_{j,f}(t) - \overline{\text{spec}}_j) \quad (\text{Equation 1})$$

where T is the number of time windows, F is the number of frequency bins, $\text{spec}_{i,f}(t)$ is the spectrogram of electrode i at time window t and frequency f , and $\overline{\text{spec}}_i$ is the mean spectrogram of electrode i .

Level of disarrangement metric (ϵ)

We selected the principal components carrying the largest fraction of variance and projected them back into the original electrode space. Unsupervised clustering was then performed using mean-shift clustering from the Scikit-learn Python library,⁴⁴ with an estimated bandwidth function (quantile = 0.45, $n_{\text{samples}} = 80$).

To quantify the level of disarray in each MEA array, we used the projection of the electrode clustering result and calculated the sparseness of clusters across the array:

$$\epsilon = \frac{1}{C} \sum_{i=1}^L \frac{n_{\text{cluster},i}}{n_{\text{electrode},i}} \quad (\text{Equation 2})$$

where C is the total number of clusters found, L is the number of lines in the array ($L = 8$), $n_{\text{cluster},i}$ is the number of clusters cohabiting on line i , and $n_{\text{electrode},i}$ is the total number of electrodes on line i (ranging from 6 to 8 depending on array geometry). Differences in ϵ between groups were assessed using the Mann–Whitney U test.

Common band determination

Inspired by the methodology of Zhang W. et al.,²⁹ we aimed to identify a common oscillatory band across the computed spectrograms using basal activity, and to verify whether that oscillation persisted during Mg^{2+} -Free and recovery conditions. For this purpose, PCA was performed from the covariance matrix of each time window over all electrodes e and frequencies f :

$$C_{ij} = \frac{1}{F} \cdot \frac{1}{E} \sum_{e=0}^E \sum_{f=1}^F (\text{Spec}_{i,e,f} - \overline{\text{Spec}}_i) (\text{Spec}_{j,e,f} - \overline{\text{Spec}}_j) \quad (\text{Equation 3})$$

where F is the total number of frequencies, E is the number of electrodes, $\text{Spec}_{i,e,f}$ is the spectrogram at time window i , electrode e , and frequency f , and $\overline{\text{Spec}}_i$ is the mean spectrogram at time window i .

We projected the first 4 principal components to evaluate the variance carried within each (see Supplementary 4). By computing the mean over extreme examples (Dyslaminated, Eulaminated) in baseline activity, we identified the frequency range where the first component varies most, and then evaluated how that range persisted across other recordings and conditions.

Aperiodic exponent fitting and bimodality

Following the literature,⁸ we evaluate the aperiodic exponent in every electrode at baseline activity, Mg²⁺-Free and recovery by fitting the Lorentzian function:

$$L = b - \log(k + F^x)$$

Where b is the broadband offset, x is the exponent and k the knee parameter, that according to Donoghue, T.⁸ and Miller, K.⁴⁵ and also applied by Gao, R.,³⁸ allows a control of the bend in the aperiodic component. F corresponds to the frequency vector in input frequencies. Knowing that when $k = 0$ the Lorentzian fits a line in log-log space.

To perform the tendency of the power log, we applied a Gaussian filter using Scipy⁴⁶ with a $\sigma = 5$ and also measured the variance respecting the logarithmic scale to approach the level of variation of spectrograms along all our electrodes shown in Figures 1 and 2.

Transfer entropy along electrode clusters

We computed transfer entropy during baseline, Mg²⁺-Free, and recovery conditions from the common LFP band identified for each cluster, where X_t denotes the LFP from cluster n and Y_t from cluster m . We used a finite set of lags $L = \{\tau_1, \tau_2, \dots, \tau_k\}$ (200 lags, range $[-100, 100]$). For each lag, the lagged versions of the time series are defined as:

$$X_t(\tau) = X_{t+\tau}, \quad Y_t(\tau) = Y_{t+\tau} \quad (\text{Equation 4})$$

We then computed the mutual information (MI) between $X_t(\tau)$ and Y_t :

$$MI_{XY}(\tau) = I(X_t(\tau); Y_t), \quad MI_{YX}(\tau) = I(Y_t(\tau); X_t) \quad (\text{Equation 5})$$

where I denotes Shannon mutual information and H Shannon entropy:

$$I(A; B) = H(A) + H(B) - H(A, B) \quad (\text{Equation 6})$$

with A and B representing different cluster sets. To compute directionality, we identified the lag that maximizes mutual information:

$$XY = \operatorname{argmax}_{\tau} MI_{XY}(\tau) \quad (\text{Equation 7})$$

The same logic was applied in the reverse direction YX . When more than two electrode clusters were found, all possible pairwise combinations were used to evaluate all directional interactions.

Electrode depth segmentation of the MEA

To investigate the relationship between electrode depth and information transfer, we reconstructed the spatial arrangement of the MEA. The array pattern was defined according to the experimental layout, with electrodes positioned in a hexagonal grid. For each electrode, x and y coordinates were computed based on grid spacing, and the vertical (y) coordinate was used to estimate relative cortical depth. Depth values were normalized to the interval $[0, 1]$, where 0 corresponds to the deepest electrodes and 1 to the most superficial.

Following normalization, electrodes were sorted by depth and divided into three layers (upper, middle, lower), each containing approximately one-third of the electrodes, yielding three partitions (A, B and C) used for subsequent analyses of directional information transfer.

Multi-set directionality computation

The directionality analysis was extended to multiple signal sets by evaluating all pairwise interactions. Each pair of sets was analyzed independently following the same time-lagged MI and permutation-testing procedure described in Section 10.7.

Directionality asymmetry index

To summarize net directional influence between two signal sets, the Directionality Asymmetry Index (DAI) was computed as:

$$DAI = \frac{TE_{X \rightarrow Y} - TE_{Y \rightarrow X}}{TE_{X \rightarrow Y} + TE_{Y \rightarrow X}} \quad (\text{Equation 8})$$

where $TE_{X \rightarrow Y}$ and $TE_{Y \rightarrow X}$ denote the total information transfer in each direction. The DAI ranges from -1 to 1 , indicating dominant directional influence or balanced bidirectional interactions.

MI of LFP components (magnitude, phase, and instantaneous frequency) during time evolution

Following the assumption the information carried in an LFP signal is distributed differently as Kayser et al.³³ has described. We decompose our LFP in the common band evaluated (0.5–30 Hz) using a discrete Hilbert Transform,⁴⁶ to obtain the phase, envelope (energy) and instantaneous frequency of every electrode. MI between every pair of electrodes was then computed using a sliding window of 15 s with 75% overlap:

$$I(X; Y) = H(X) + H(Y) - H(X, Y) \quad (\text{Equation 9})$$

where X and Y represent the signals of two different electrodes across the MEA. For each electrode, the maximum MI value across all pairwise interactions was retained, and this was computed for every condition: Baseline, Mg^{2+} -Free (3 samples), and Recovery (2 samples). This metric does not inform directionality or causation; it was used here as a measure of the total information carried under each condition.

Pairwise dissimilarity evaluation and multidimensional scaling

We computed temporal dissimilarity matrices D^j , previously obtained from MI temporal sliding windows of every LFP component, where each entry D^j_{ab} quantifies the Euclidean distance between the a -th entity at time j and the b -th entity at time $j + 1$, decomposing the system's temporal evolution into interpretable pairwise dissimilarities:

$$D^j_{ab} = \sqrt{\sum_{k=1}^d (x^j_{a,k} - x^{j+1}_{b,k})^2} \quad (\text{Equation 10})$$

where k represents the feature/dimension index in each sample vector, with $a = 1, \dots, m$ and $b = 1, \dots, n$. MDS was then applied to the reduced vectors D^j_{ab} to assess how activity differed from baseline across Mg^{2+} -Free and recovery conditions.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were performed in Python using SciPy⁴⁶ and Scikit-learn,⁴⁴ unless otherwise stated. A significance threshold of $\alpha = 0.05$ was applied throughout.

Quality control

Signal quality was verified at two stages. First, the logarithmic power spectrum of each electrode was inspected visually and compared across recordings. Second, electrode traces deviating beyond ± 2 standard deviations in the principal components identified during dimensionality reduction (Section 4.5.3) were flagged and excluded from further analysis. Sample sizes after quality control: Eulaminated $n = 3$; Dyslaminated $n = 5$.

Aperiodic exponent thresholding and bimodality

To identify a data-driven boundary separating the two groups, kernel density estimation (KDE) was applied independently to each distribution using a Gaussian kernel. The summed density function was inspected for local minima, and the intersection of the two KDEs was computed as the point where the signed difference between densities changed sign. Both approaches converged on a threshold of 0.6, consistent with the visual valley observed in the KDE plot.

Classification performance at this threshold was quantified using sensitivity (true positive rate for Dyslaminated), specificity (true negative rate for Eulaminated), overall accuracy, and balanced accuracy (reported to account for group size imbalance).

To assess whether classification accuracy was disproportionately influenced by individual observations, a leave-one-out (LOO) analysis was performed for each group: at each iteration, a single observation was removed and all metrics were recomputed. The standard deviation of accuracy, sensitivity, and specificity across LOO iterations served as an index of sample susceptibility.

Bootstrap resampling ($n = 1,000$ iterations, with replacement) was used to derive 95% confidence intervals around the accuracy estimate. The degree of bimodality was quantified using the bimodal coefficient statistic.⁴⁷ A simple ordinary least-squares (OLS) linear regression was then fitted between the bimodality coefficient and ϵ at baseline to characterize the relationship between spectral heterogeneity and spatial disarrangement.

Multidimensional scaling and condition comparison

Temporal dissimilarity matrices were subjected to classical (metric) MDS to obtain low-dimensional embeddings of electrode-activity trajectories across conditions (Baseline, Mg^{2+} -Free, Recovery). The first two MDS dimensions were retained, and a 95% confidence ellipse was fitted to each condition's point cloud to characterize trajectory dispersion. Differences in spread between conditions and LFP components (magnitude, phase, instantaneous frequency) were evaluated using an independent-samples Student's t test applied to the ellipse semi-axis lengths.

Group comparison of the disarrangement metric (ϵ)

Differences in ϵ between Eulaminated ($n = 3$) and Dyslaminated ($n = 5$) preparations were assessed using the two-sample Mann–Whitney U test, a non-parametric rank-based test chosen because of the small and unequal group sizes and the absence of normality assumptions.

Permutation test for correlation between ϵ and the directionality asymmetry index

To assess whether the DAI was significantly associated with ϵ , we employed a non-parametric permutation test. The observed Pearson correlation coefficient r between the two variables was first computed. A null distribution was generated by randomly permuting the DAI values 10,000 times while keeping ϵ fixed, and recomputing r for each permutation. The p -value was defined as:

$$p = \frac{1}{N} \sum_{k=1}^N \mathbf{1}(|r_k^*| \geq |r_{\text{obs}}|), \quad N = 10,000 \quad (\text{Equation 11})$$

The observed correlation was $r = 0.25$ ($p = 0.23$), indicating no statistically significant association between the two measures under the conditions studied.

Permutation-based significance testing of mutual information

To determine whether the observed MI at a given lag exceeded chance levels, a permutation null distribution was constructed independently for each lag τ . Specifically, $N = 1,000$ surrogate time series $Y_t^{(n)}$ were generated by randomly shuffling the original Y_t (and equivalently X_t for the reverse direction). MI was recomputed for each surrogate, yielding a null distribution. Only lag values for which the observed MI exceeded the 95th percentile of the null distribution ($p < 0.05$, one-tailed) were retained for subsequent directionality analyses.

Comparison of directional information flow

For each combination of cluster pair, experimental group, and activity condition, the distribution of mean MI values in the forward direction was compared with that in the reverse direction using a two-sided Mann–Whitney U test. This non-parametric test was selected because (i) normality of mean MI distributions could not be assumed given our sample sizes, and (ii) the number of electrodes per direction within each stratum was unequal.

Multiple comparison correction

Statistical tests were performed across all combinations of cluster pairs, experimental groups, and activity conditions, yielding $K = 18$ simultaneous comparisons. p -values were adjusted using the Bonferroni procedure. Statistical significance was declared at $\alpha = 0.05$ after correction, with the annotation scheme: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p \geq 0.05$ (ns).

Effect size

To quantify the magnitude of directional asymmetry independently of sample size, the rank-biserial correlation coefficient r was computed as an effect size measure for the Mann–Whitney test:

$$r = 1 - \frac{2U}{n_1 n_2} \quad (\text{Equation 12})$$

Effect sizes were interpreted as: $|r| < 0.3$ (small), $0.3 \leq |r| < 0.5$ (medium), and $|r| \geq 0.5$ (large). Positive values of r indicate higher mean MI in the forward direction; negative values indicate dominance of the reverse direction.